General Overview of
Secondary Metabolism

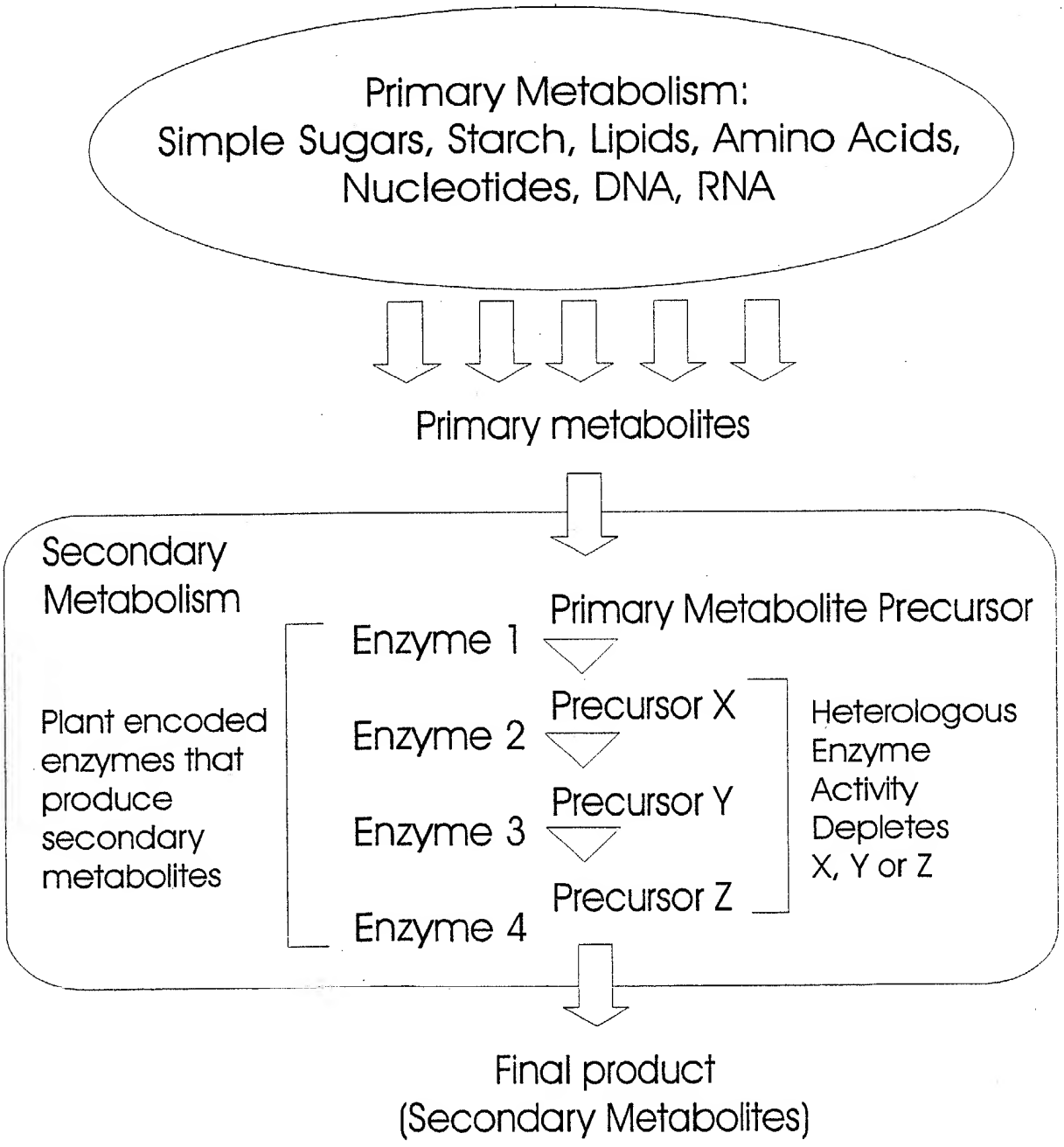


FIG. 1

General Phenylpropanoid Metabolism

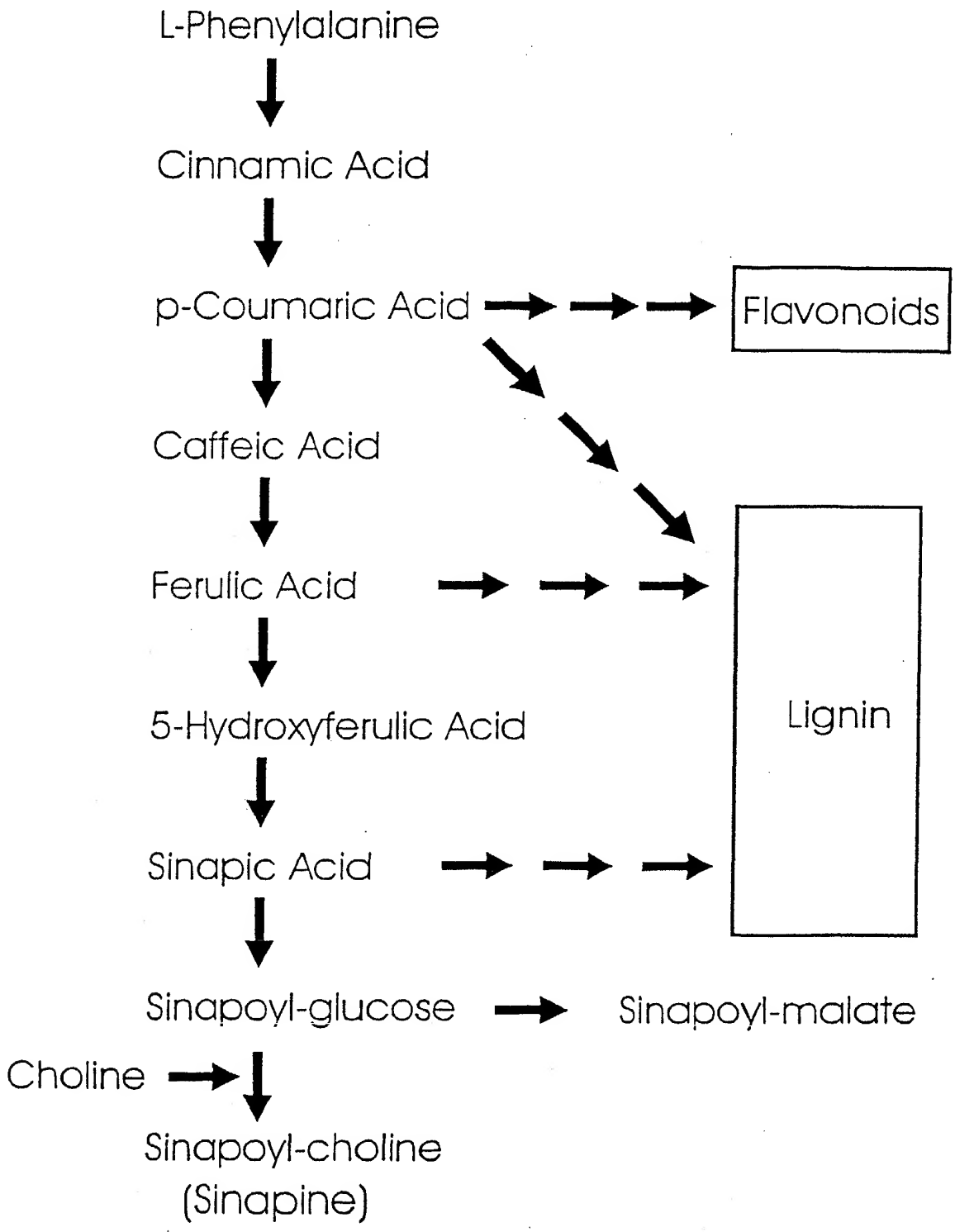


FIG. 2

Sinapine in *Brassica napus* Seeds

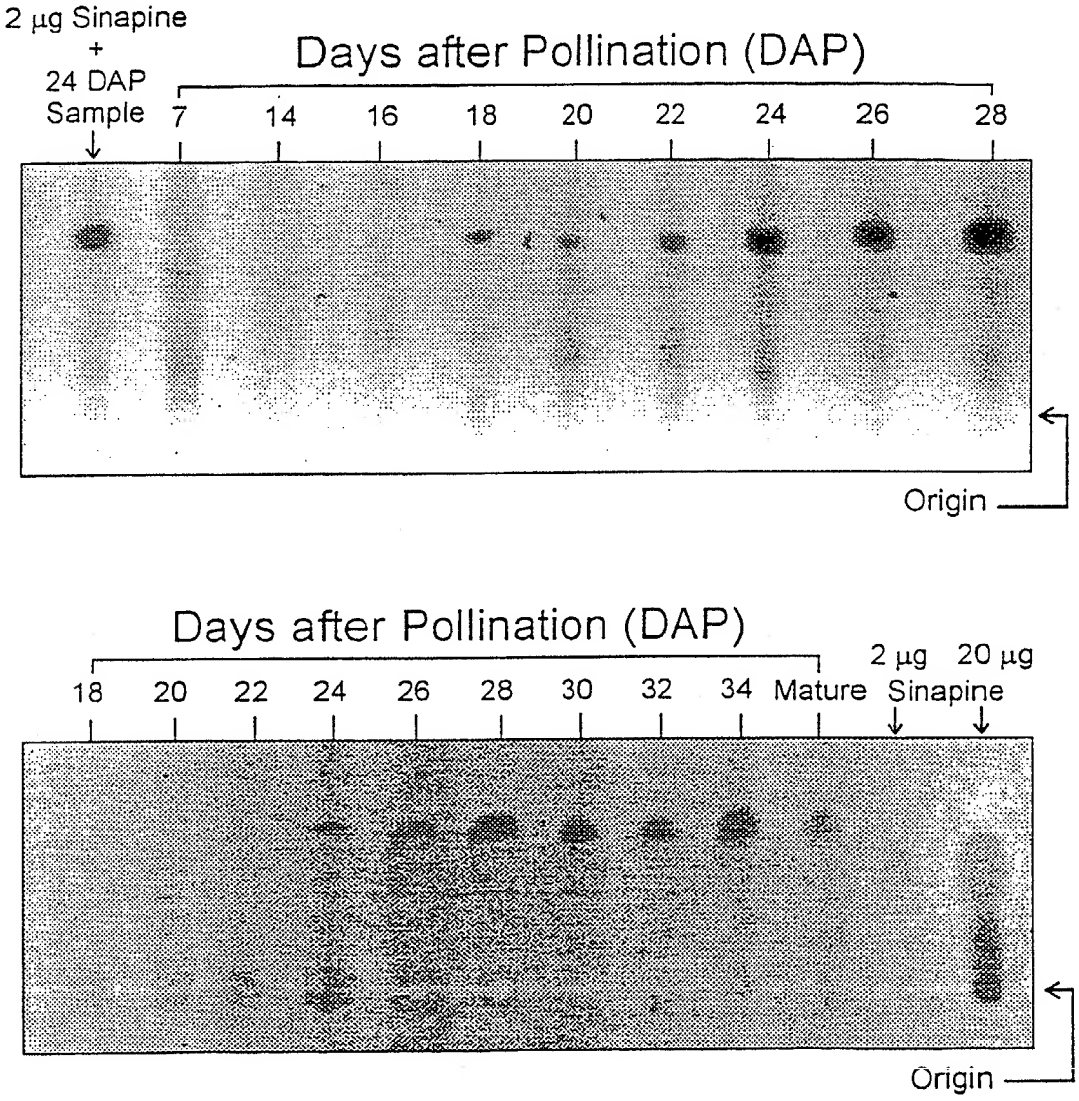


FIG. 3

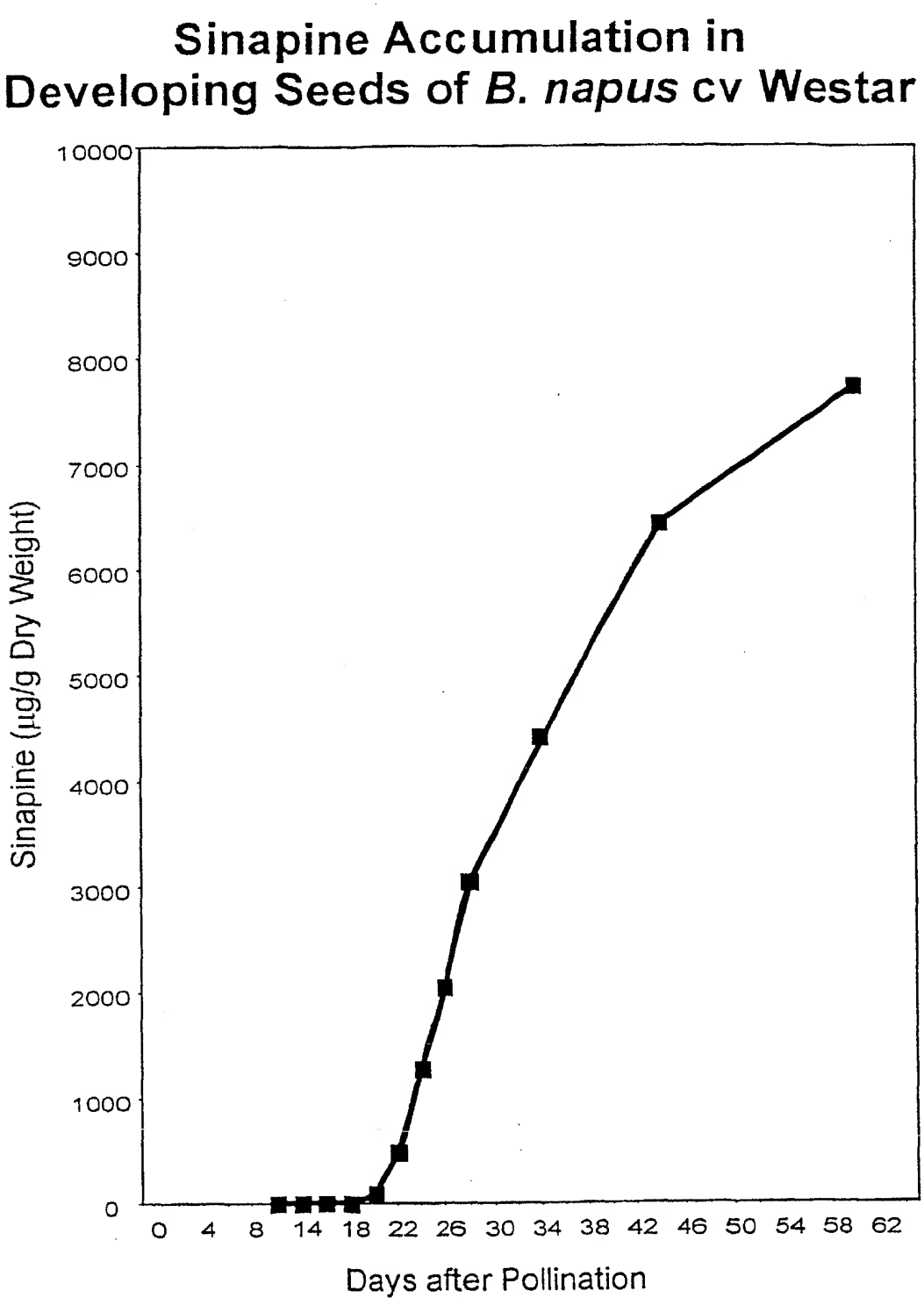

Sinapine Accumulation in
Developing Seeds of B. napus cv Westar
Sinapine (µg/g Dry Weight)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0 4 8 14 18 22 26 30 34 38 42 46 50 54 58 62
Days after Pollination

FIG. 4

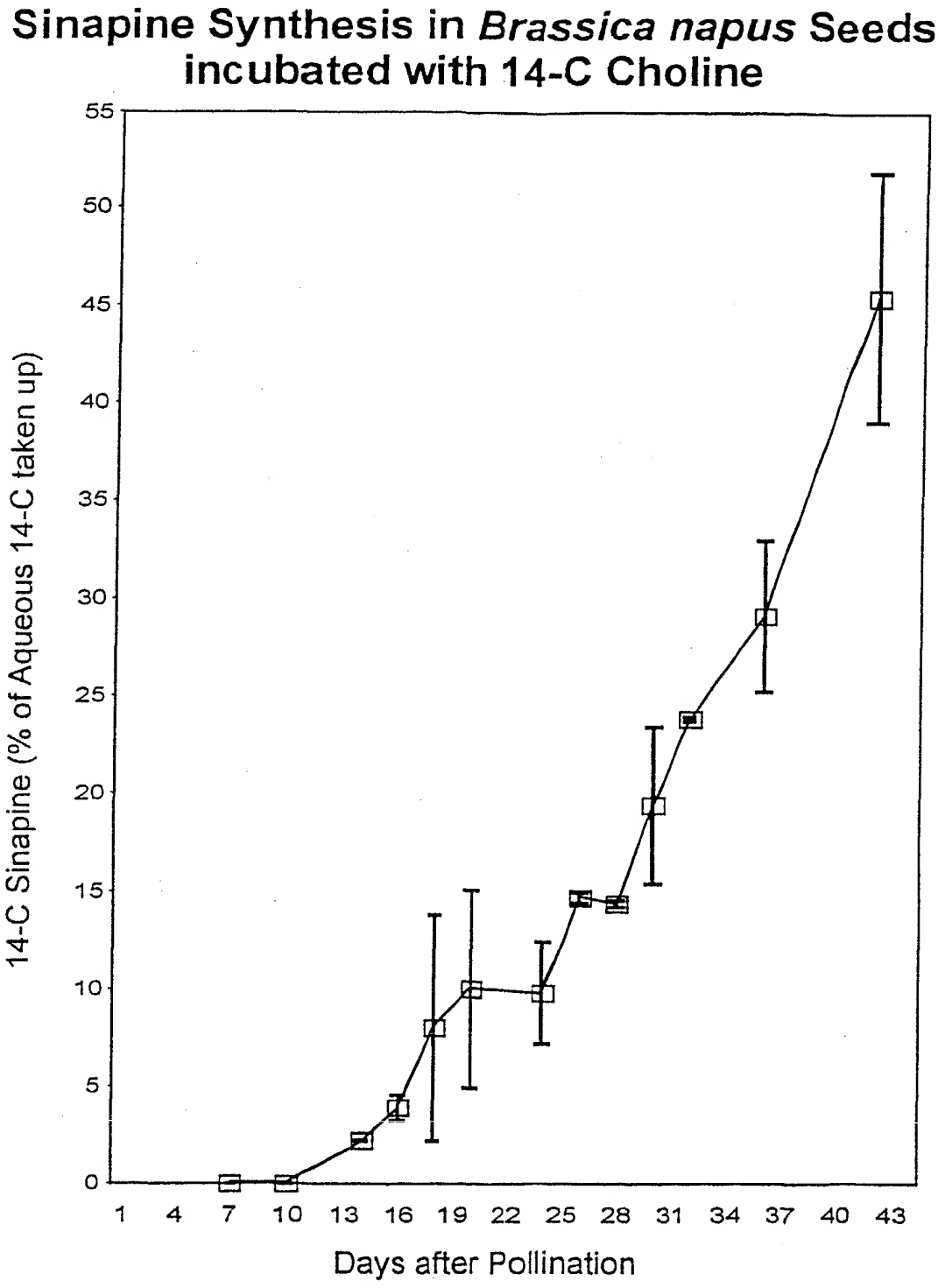

Sinapine Synthesis in Brassica napus Seeds
incubated with 14-C Choline
14-C Sinapine (% of Aqueous 14-C taken up)
0
5
10
15
20
25
30
35
40
45
50
55
1
4
7
10
13
16
19
22
25
28
31
34
37
40
43
Days after Pollination

FIG. 5

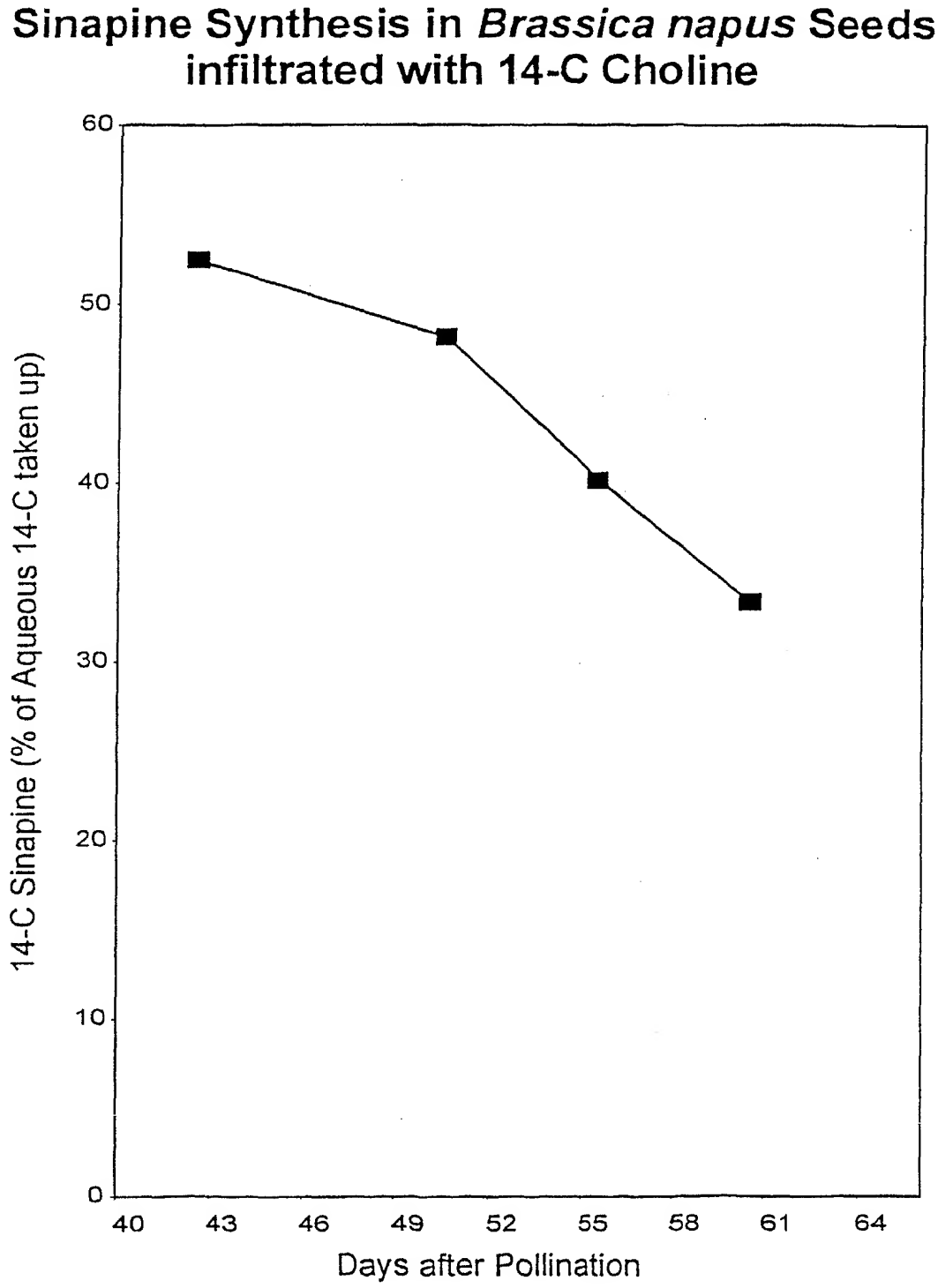

Sinapine Synthesis in Brassica napus Seeds infiltrated with 14-C Choline
14-C Sinapine (% of Aqueous 14-C taken up)
60
50
40
30
20
10
0
40
43
46
49
52
55
58
61
64
Days after Pollination

FIG. 6

Location of Radiolabelled Sinapine in *B. napus* seed

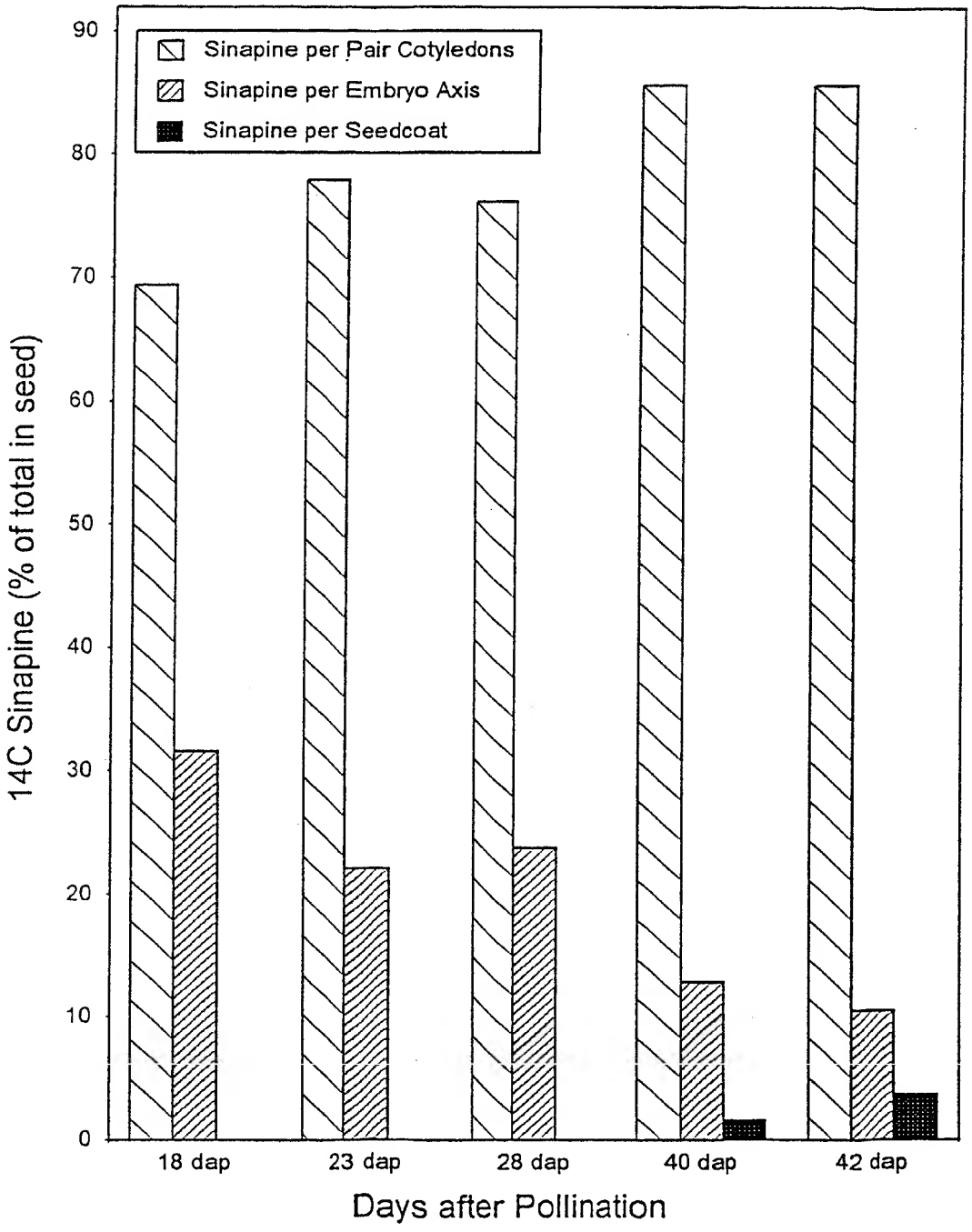


FIG.7

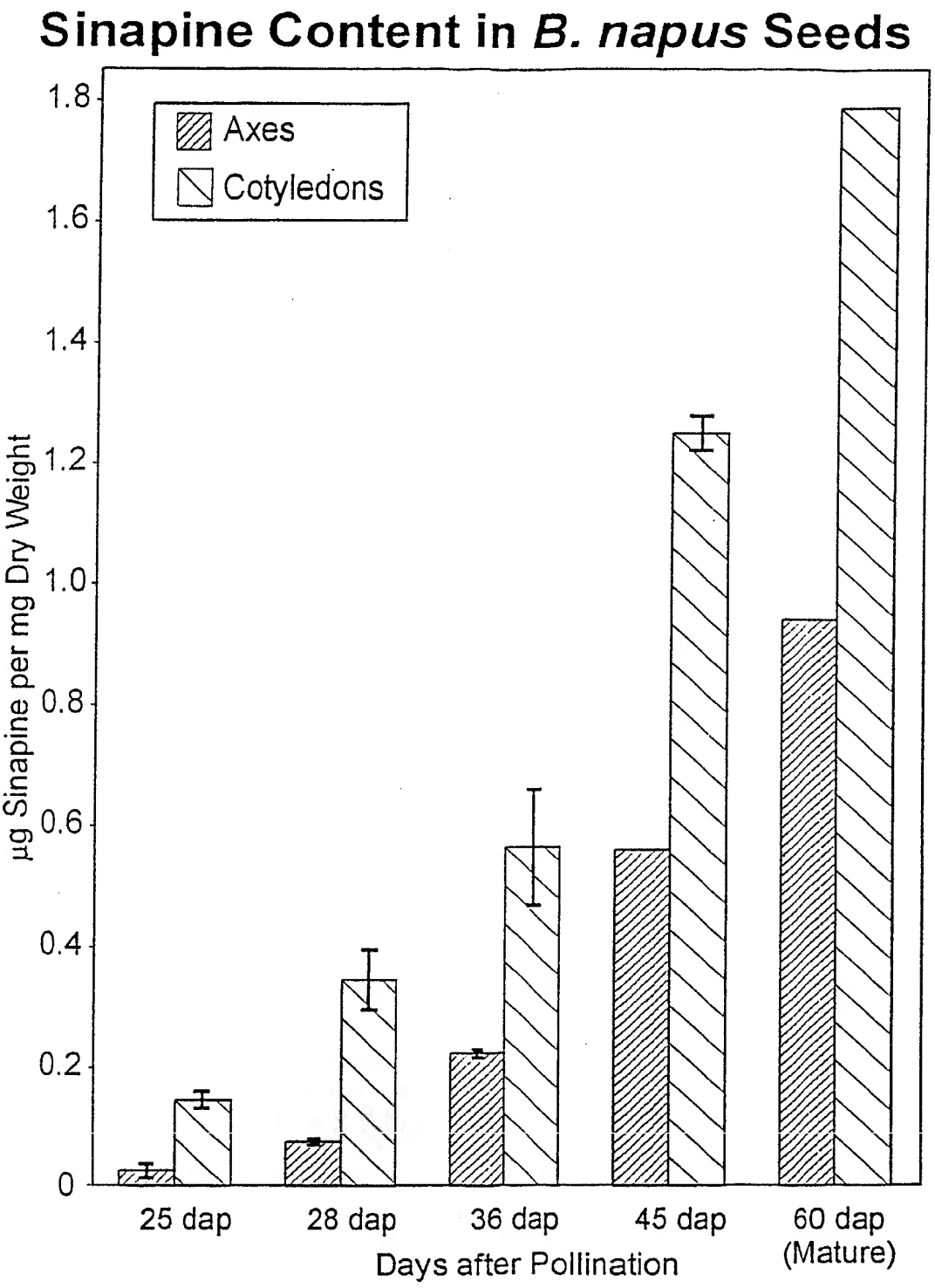

Sinapine Content in B. napus Seeds
Axes
Cotyledons
µg Sinapine per mg Dry Weight
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
25 dap
28 dap
36 dap
45 dap
60 dap
(Mature)
Days after Pollination

FIG. 8

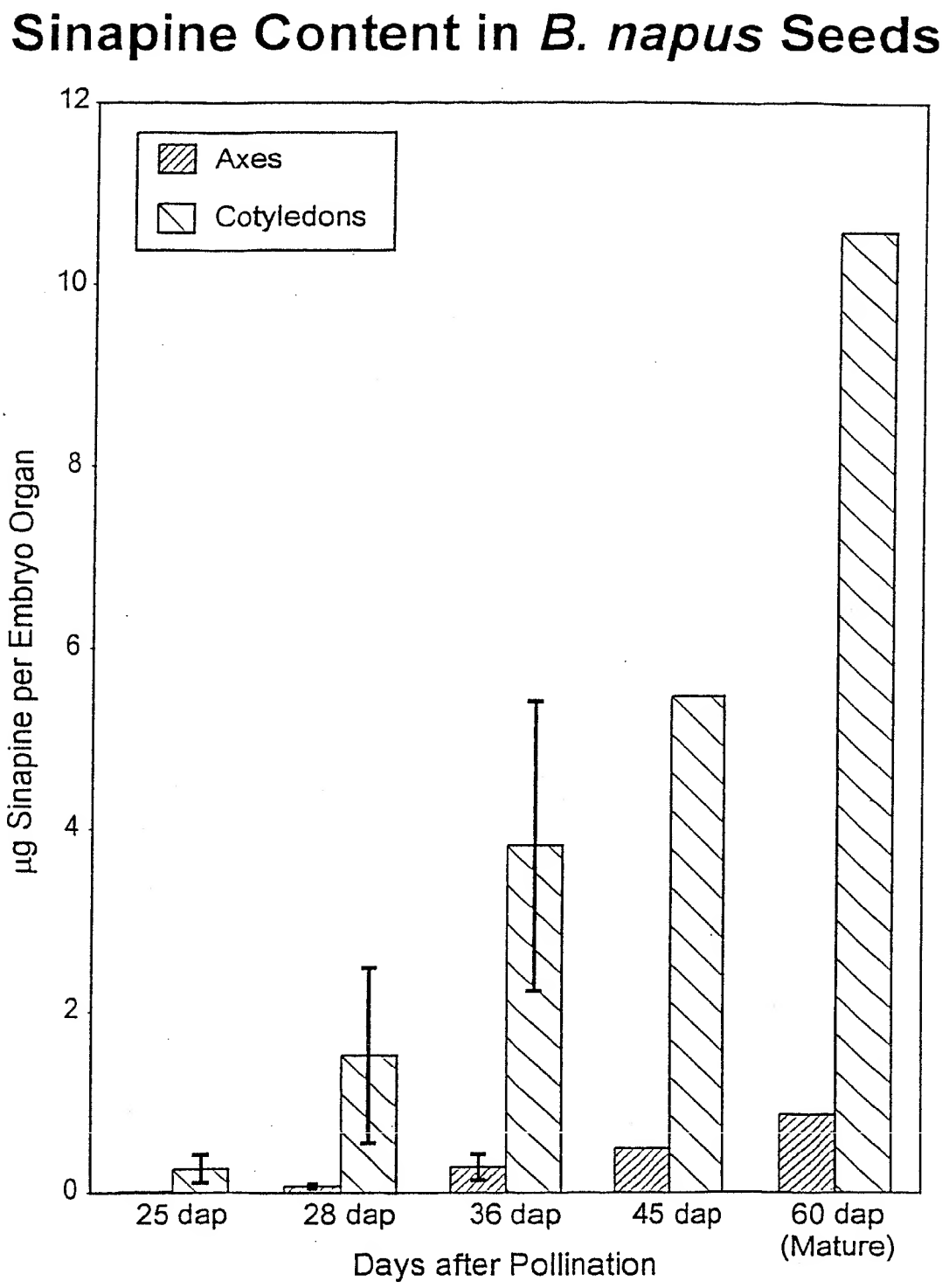

Sinapine Content in B. napus Seeds
Axes
Cotyledons
µg Sinapine per Embryo Organ
0
2
4
6
8
10
12
25 dap
28 dap
36 dap
45 dap
60 dap
(Mature)
Days after Pollination

FIG. 9

```
   1  ATGCACATCG ACAACGTCGA AAACCTCAAC GACCGCGAGT TCGACTACAT
  51  CATCATCGGC GGCGGTTCCG CCGGAGCGGC AGTCGCCGCC CGCCTGAGCG
 101  AGGAGCCCAC CGTGTCCGTG GCGCTGGTGG AGGCCGGCCC GGACGACCGC
 151  GGCGTTCCCG AGGTACTGCA GCTCGACCGC TGGATGGAGC TGCTGGAATC
 201  CGGCTACGAC TGGGACTACC CGATCGAACC GCAGGAGAAC GGCAACTCCT
 251  TCATGCGCCA CGCCCGCGCG AAGATCATGG GTGGCTGCTC CAGCCACAAC
 301  TCCTGCATCG CCTTCTGGGC CCCGCGCGAA GACCTGGACG AGTGGGAGTC
 351  CAAGTACGGC GCCACCGGCT GGAACGCTGA GTCCGCCTGG CCGCTGTACC
 401  AGCGGCTGGA GACCAACGAG GACGCCGGCC CGGACGCGCC GCACCACGGC
 451  GACTCAGGCC CGGTGCACCT GATGAACGTG CCCCCGGCGG ACCCCGCCGG
 501  CGTCGCACTC CTGGACGCCT GCGAACAGGC AGGCATTCCG CGCGCGAAGT
 551  TCAACACCGG CACCACCGTG ATCAATGGCG CCAACTTTTT CCAGATCACA
 601  CGCCGCGCGG ACGGCACCCG TTCCTCCAGC TCGGTCTCCT ACATCCACCC
 651  GATCATCGAG CGCGGGAACT TCACCCTGCT GACCGGGTTG CGCGCCCGGC
 701  AACTGGTGTT CGACGCGGAC AAGCGCTGCA CCGGCGTCGA CGTTGTGGAC
 751  TCGGCGTTCG GCCGGACTCA CCGGCTCTCC GCGCGTTGCG AGGTCATCCT
 801  GTCCACCGGC GCCATTGACT CGCCTAAGCT GCTCATGCTC TCCGGCATCG
 851  GCCCCGCCGC GCACCTCGCC GAGCACGGCG TCGAGGTCCT GGTCGACTCC
 901  CCCGGTGTCG GCGAGCACCT GCAGGACCAC CCCGAAGGCG TCGTCCAGTT
 951  CGAGGCCAAG CAGCAGATGG TGCAGACTTC GACGCAGTGG TGGGAGATCG
1001  GCATCTTCAC CCCCACCGAG AACGGCCTGG ACCGCCCGGA CCTGATGATG
1051  CACTACGGCT CCGTCCCGTT CGACATGAAC ACCCTGCGGT ACGGCTACCC
1101  CACCACGGAG AACGGCTTCA GCCTCACGCC GAACGTCACG CACGCCCGCT
1151  CCCGCGGCAC CGTCCGGCTG CGCAGCCGCG ACTTCCGCGA CAAGCCCGCC
1201  GTCGACCCGC GGTACTTCAC TGATCCGGAG GGCCACGACA TGCGCGTCAT
1251  GGTGGCCGGC ATCCGCAAGG CCCGTGAAAT CGCCGCCCAG CCTGCCATGG
1301  CCGAATGGAC CGGCCGCGAG CTCTCGCCCG GCACCGAGGC GCAGACCGAC
```

FIG. 10A

```
1351  GAGGAACTGC AGGACTACAT CCGCAAGACG CACAACACCG TTTACCACCC

1401  CGTCGGCACC GTCCGCATGG GACCAGCCGA CGACGACATG TCGCCGCTCG

1451  ACCCCGAGCT GCGGGTGAAG GGCGTGACCG GCCTGCGCGT CGCCGATGCC

1501  TCTGTCATGC CTGAACACGT CACGGTCAAT CCCAACATCA CCGTCATGAT

1551  GATCGGCGAA CGCTGCGCCG ACCTCATCCG CGCCAGCCGG ACCGGCGAAA

1601  CAACGACGGC GGAGGCGGAG CTCAGCGCGT CCCTCGCCTG A
```

FIG. 10B

Predicted amino acid sequence of choline oxidase open frame.

```
  1  MHIDNVENLN DREFDYIIIG GGSAGAAVAA RLSEEPTVSV ALVEAGPDDR
 51  GVPEVLQLDR WMELLESGYD WDYPIEPQEN GNSFMRHARA KIMGGCSSHN
101  SCIAFWAPRE DLDEWESKYG ATGWNAESAW PLYQRLETNE DAGPDAPHHG
151  DSGPVHLMNV PPADPAGVAL LDACEQAGIP RAKFNTGTTV INGANFFQIT
201  RRADGTRSSS SVSYIHPIIE RGNFTLLTGL RARQLVFDAD KRCTGVDVVD
251  SAFGRTHRLS ARCEVILSTG AIDSPKLLML SGIGPAAHLA EHGVEVLVDS
301  PGVGEHLQDH PEGVVQFEAK QQMVQTSTQW WEIGIFTPTE NGLDRPDLMM
351  HYGSVPFDMN TLRYGYPTTE NGFSLTPNVT HARSRGTVRL RSRDFRDKPA
401  VDPRYFTDPE GHDMRVMVAG IRKAREIAAQ PAMAEWTGRE LSPGTEAQTD
451  EELQDYIRKT HNTVYHPVGT VRMGPADDDM SPLDPELRVK GVTGLRVADA
501  SVMPEHVTVN PNITVMMIGE RCADLIRASR TGETTTAEAE LSASLA*
```

FIG. 11

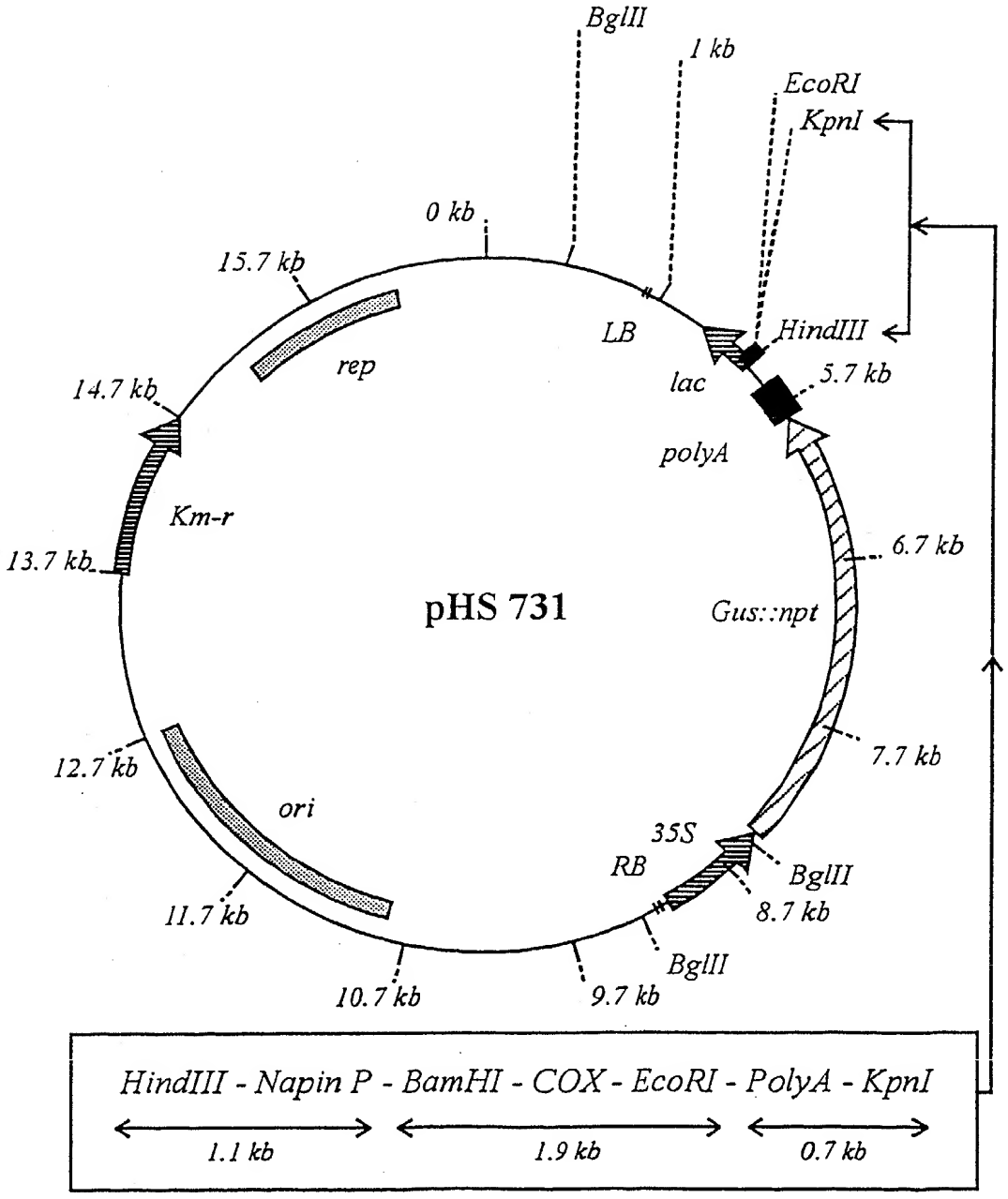

BglII
1 kb
EcoRI
KpnI
0 kb
15.7 kb
LB
HindIII
rep
lac
5.7 kb
14.7 kb
polyA
Km-r
6.7 kb
13.7 kb
pHS 731
Gus::npt
7.7 kb
12.7 kb
ori
35S
RB
BglII
8.7 kb
11.7 kb
BglII
10.7 kb
9.7 kb
HindIII - Napin P - BamHI - COX - EcoRI - PolyA - KpnI
1.1 kb
1.9 kb
0.7 kb

FIG. 12

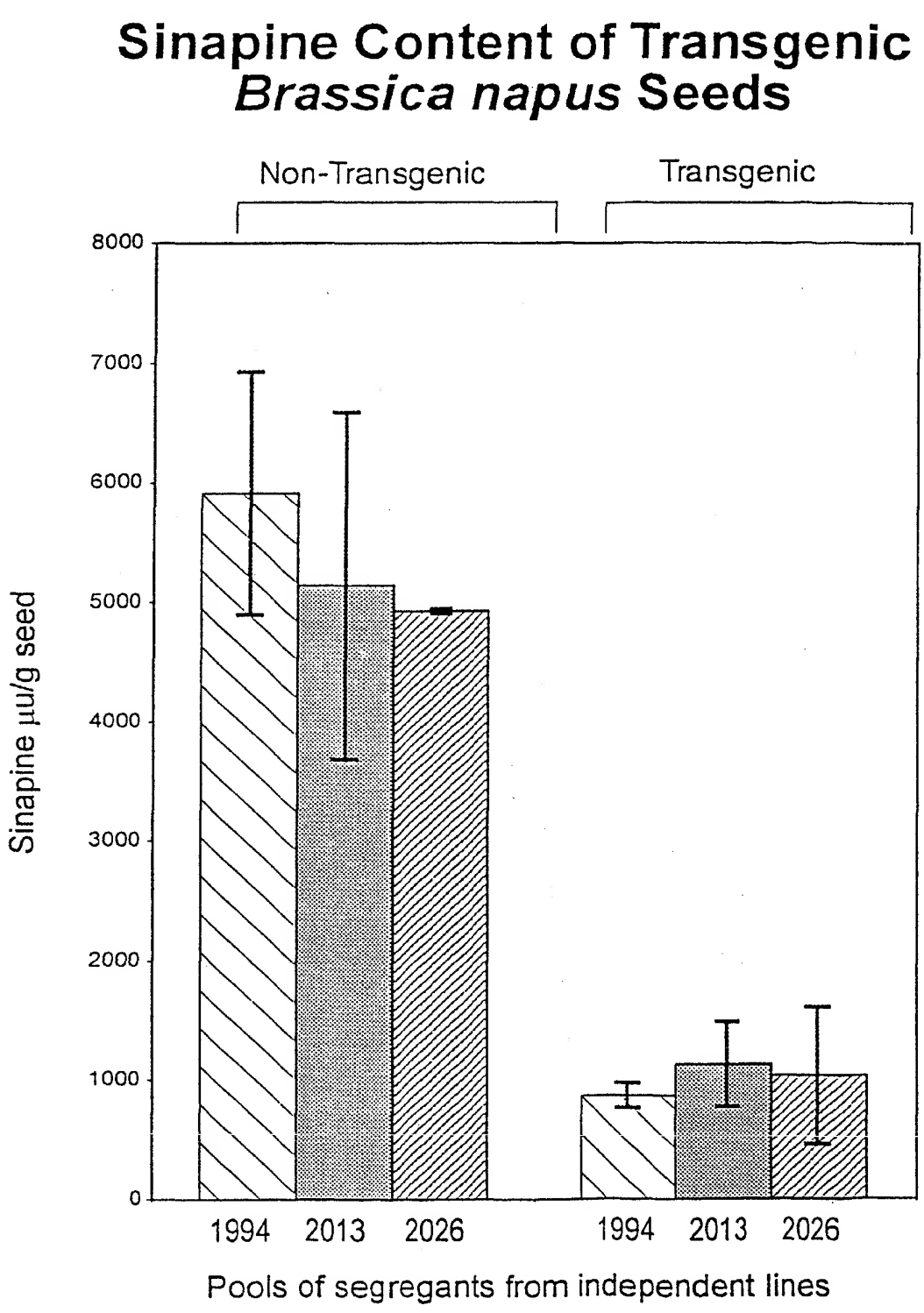

Sinapine Content of Transgenic
Brassica napus Seeds
Non-Transgenic
Transgenic
8000
7000
6000
5000
4000
3000
2000
1000
0
Sinapine µu/g seed
1994
2013
2026
1994
2013
2026
Pools of segregants from independent lines

FIG. 13

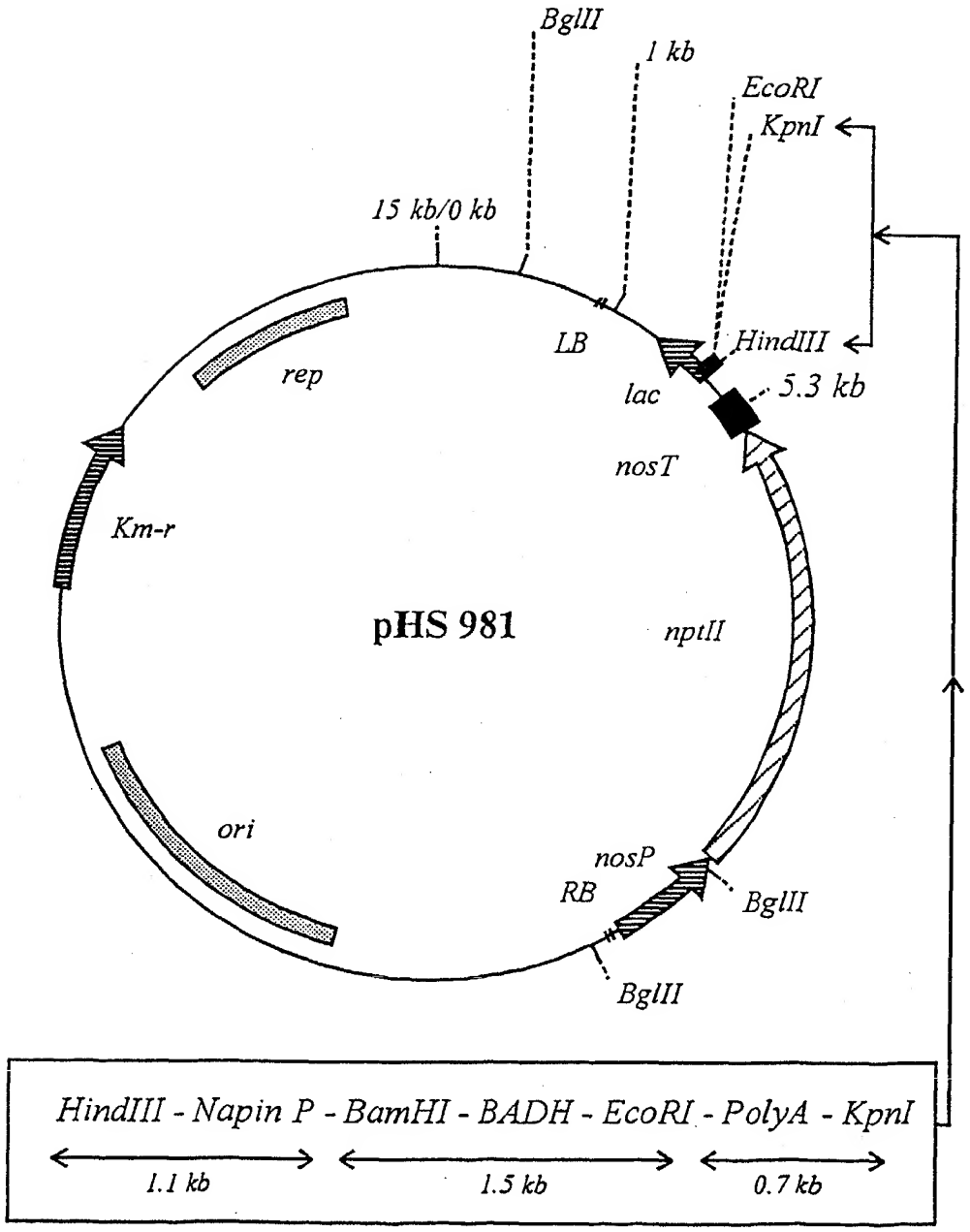

BglII
1 kb
EcoRI
KpnI
15 kb/0 kb
LB
HindIII
5.3 kb
lac
rep
nosT
Km-r
pHS 981
nptII
ori
nosP
RB
BglII
BglII
HindIII - Napin P - BamHI - BADH - EcoRI - PolyA - KpnI
1.1 kb
1.5 kb
0.7 kb

FIG. 14

Reduction of Sinapine in Cox and Cox/BaDH lines

Plant line	Number of plants analyzed	Plant Genotype	Sinapine Absorbance Units	Sinapine Levels (Control = 100%)
2026 X 1534	15	Cox + Badh	13,878	13.6%
2013	23	Cox	19,874	19.5%
1994	26	Cox	23,344	22.9%
2026	27	control	101,856	100%

FIG. 15

Total Soluble Phenolic Content in Transgenic Lines

Plant line	Number of plants analyzed	Plant Genotype	Soluble Phenolic Absorbance Units	Soluble Phenolic Levels (Control = 100%)
2026 X 1534	15	Cox + Badh	103,828	69.0%
2013	23	Cox	98,222	78.0%
1994	26	Cox	97,370	77.3%
2026	27	control	125,882	100%

FIG. 16

```
1    ATGGACCAAT TCGTGGGTCT CCACATGATC TACACATACG AGAACGGTTG
51   GGAGTACGAA ATCTACATCA AGAACGACCA CACAATCGAC TACCGTATCC
101  ACAGTGGTAT GGTGGGTGGT AGGTGGGTGA GGGACCAAGA GGTGAACATC
151  GTGAAGCTCA CAAAGGGTGT GTACAAGGTG AGCTGGACAG AGCCAACAGG
201  TACAGACGTG AGCCTCAACT TCATGCCAGA GGAGAAGAGG ATGCACGGTG
251  TGATCTTCTT CCCAAAGTGG GTGCACGAGA GGCCAGACAT CACAGTGTGC
301  TACCAAAACG ACTACATCGA CCTCATGAAG GAGAGCAGGG AGAAGTACGA
351  GACATACCCA AAGTACGTGG TGCCAGAGTT CGCTGACATC ACATACATCC
401  ACCACGCTGG AGTGAACGAC GAGACAATCA TCGCTGAgGC TCCATACGAg
451  GGTATGACAG ACGAgATCAG GGCTGGTAgG AAG
```

FIG. 17

```
1   MDQFVGLHMI YTYENGWEYE IYIKNDHTID YRIHSGMVGG RWVRDQEVNI
51  VKLTKGVYKV SWTEPTGTDV SLNFMPEEKR MHGVIFFPKW VHERPDITVC
101 YQNDYIDLMK ESREKYETYP KYVVPEFADI TYIHHAGVND ETIIAEAPYE
151 GMTDEIRAGR K
```

FIG. 18

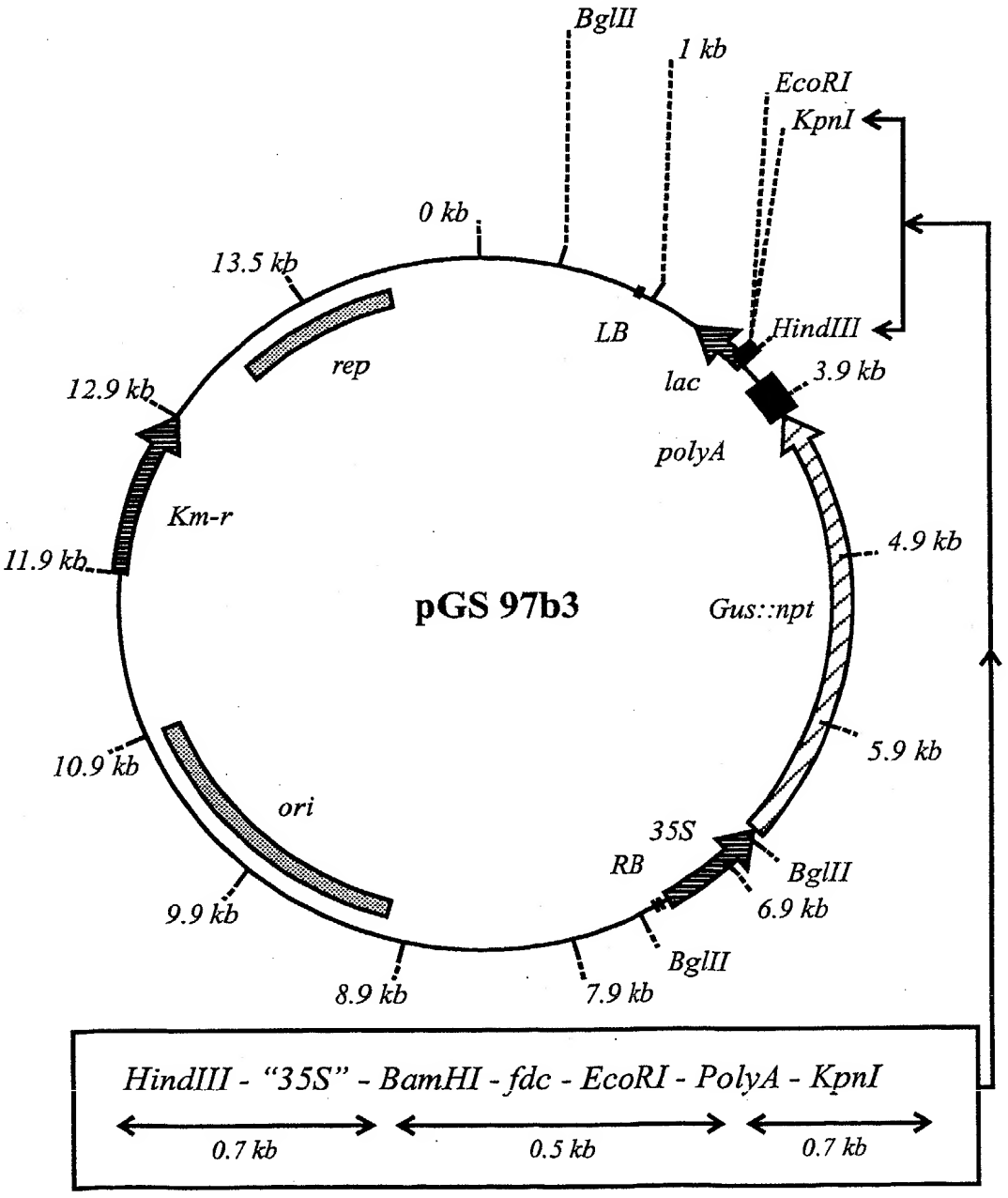

BglII
1 kb
EcoRI
KpnI
0 kb
13.5 kb
LB
HindIII
rep
lac
3.9 kb
12.9 kb
polyA
Km-r
4.9 kb
11.9 kb
pGS 97b3
Gus::npt
5.9 kb
10.9 kb
ori
35S
RB
BglII
6.9 kb
9.9 kb
BglII
8.9 kb
7.9 kb
HindIII - "35S" - BamHI - fdc - EcoRI - PolyA - KpnI
0.7 kb
0.5 kb
0.7 kb

FIG. 19

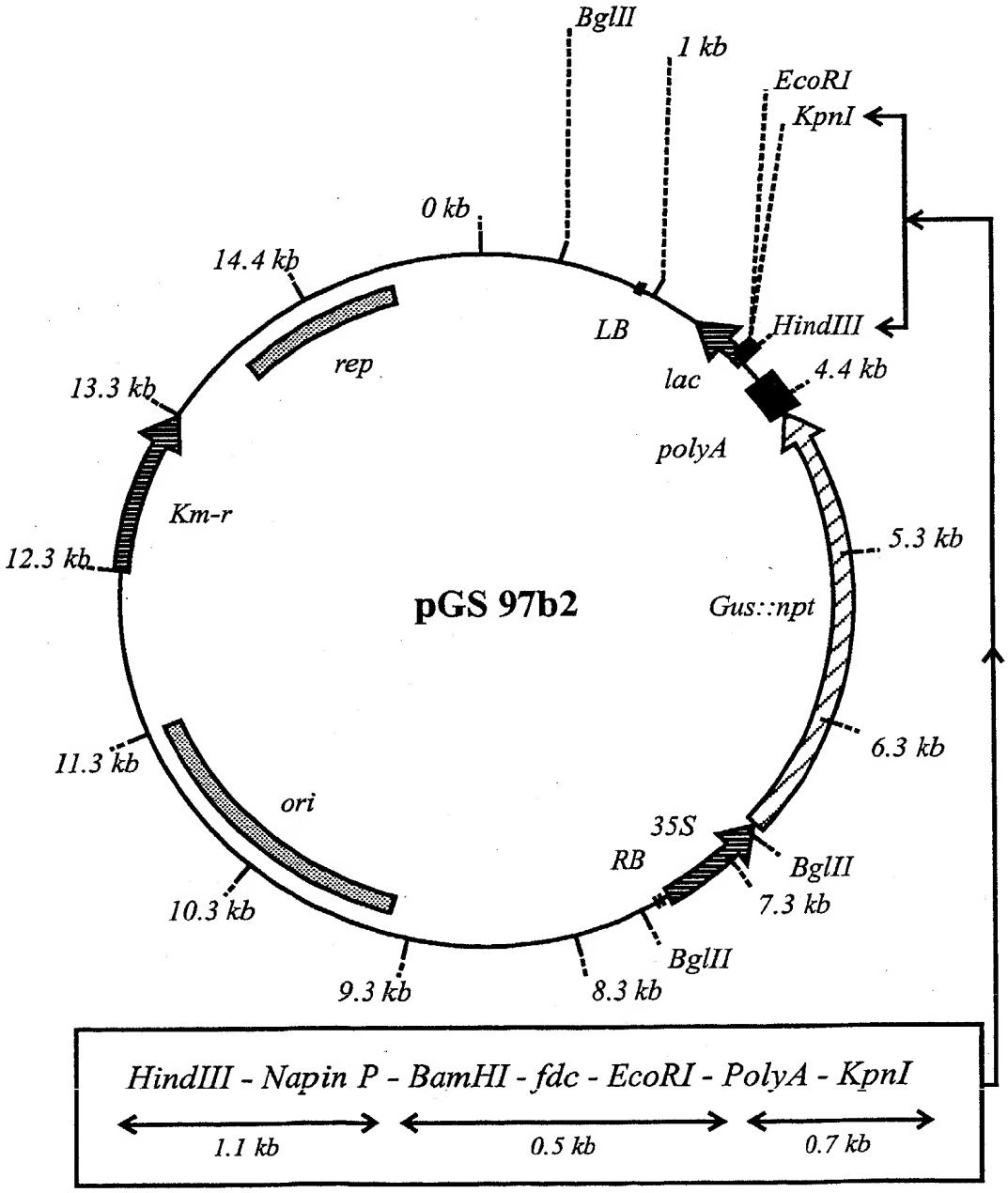

BglII
1 kb
EcoRI
KpnI
0 kb
14.4 kb
LB
HindIII
rep
lac
4.4 kb
13.3 kb
polyA
Km-r
5.3 kb
12.3 kb
pGS 97b2
Gus::npt
6.3 kb
11.3 kb
ori
35S
RB
BglII
7.3 kb
10.3 kb
BglII
9.3 kb
8.3 kb
HindIII - Napin P - BamHI - fdc - EcoRI - PolyA - KpnI
1.1 kb
0.5 kb
0.7 kb

FIG. 20

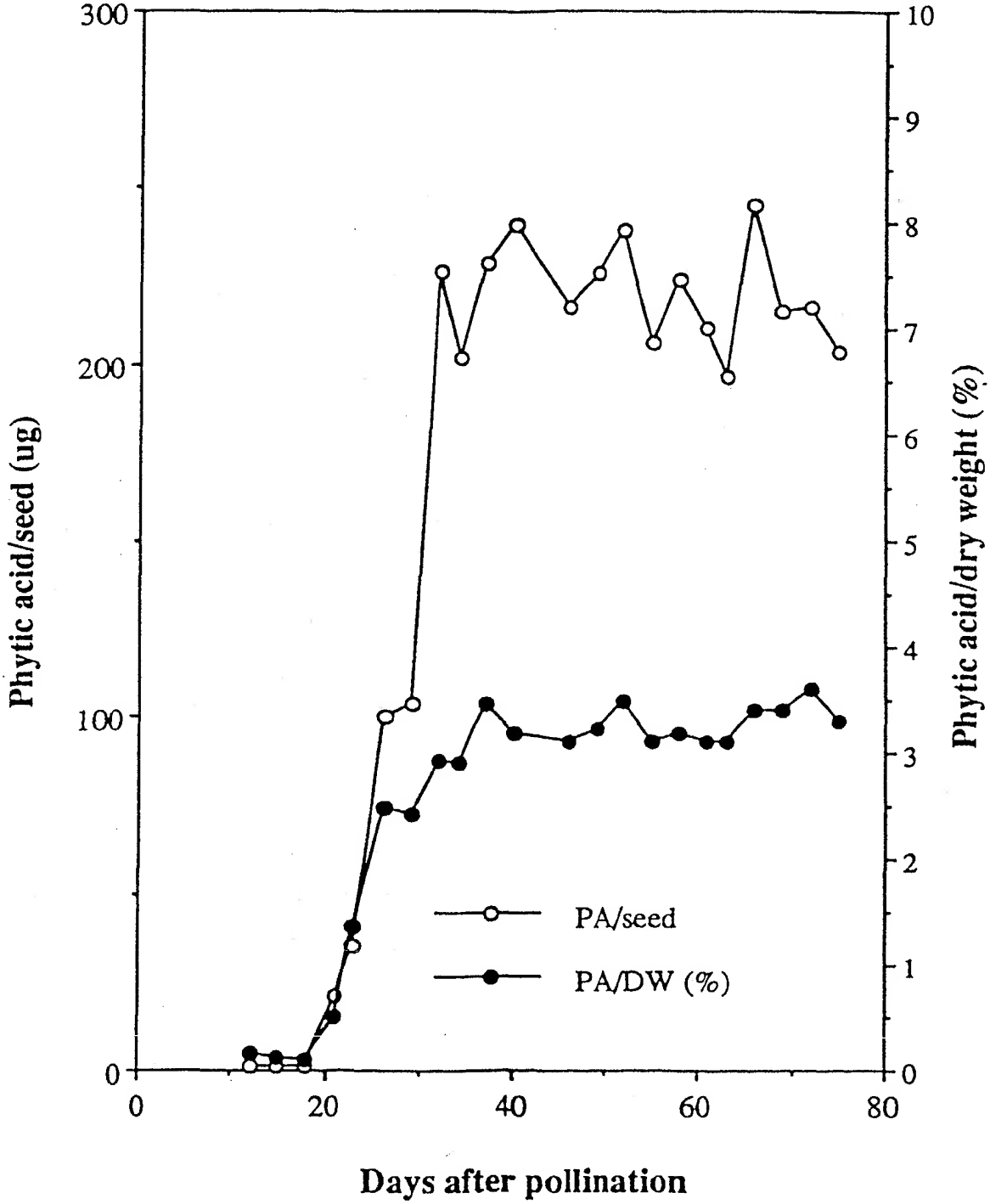

300
200
100
0
0
20
40
60
80
10
9
8
7
6
5
4
3
2
1
0
Phytic acid/seed (ug)
Phytic acid/dry weight (%)
Days after pollination
PA/seed
PA/DW (%)

FIG. 21

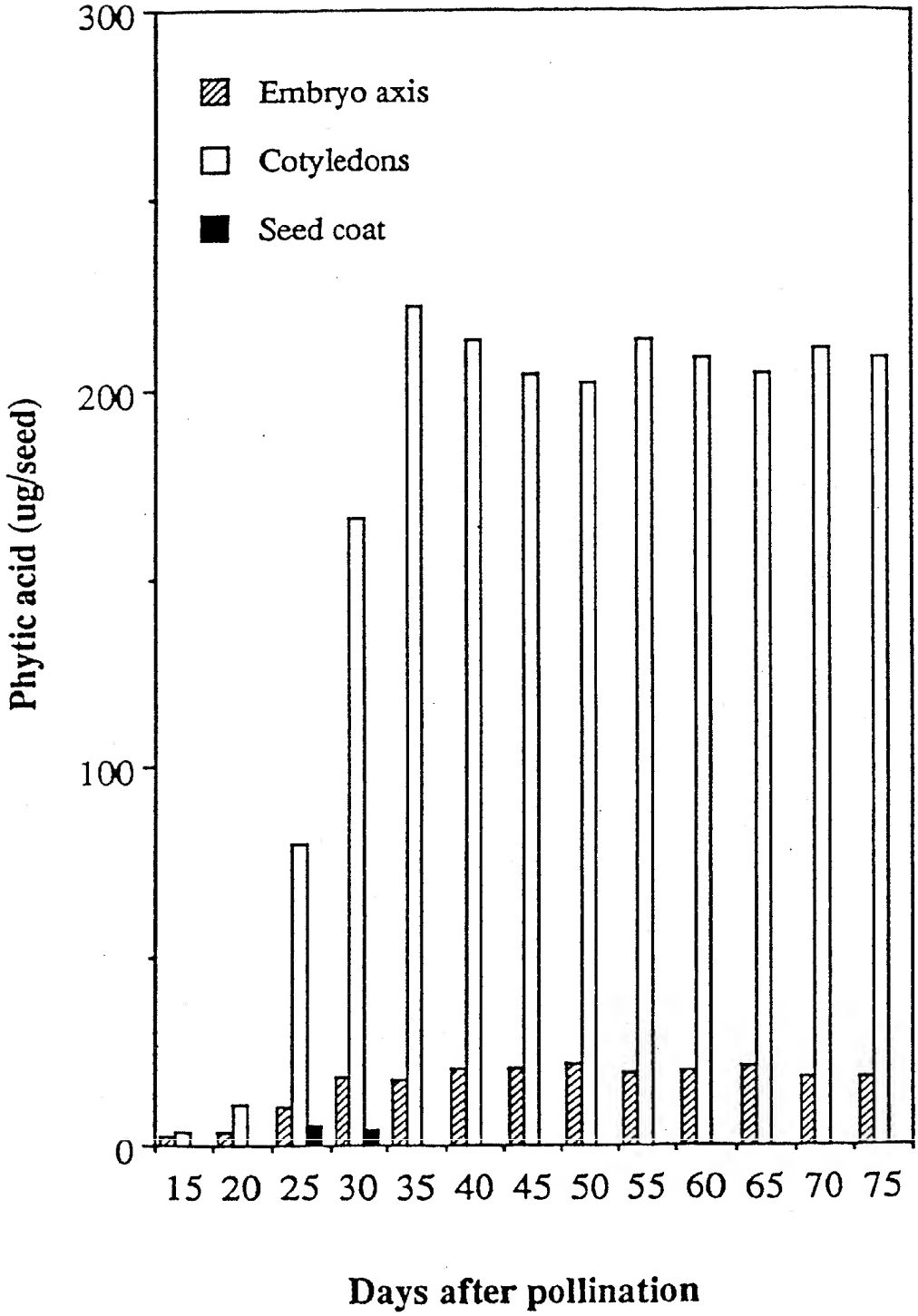

300
200
100
0
Embryo axis
Cotyledons
Seed coat
Phytic acid (ug/seed)
15 20 25 30 35 40 45 50 55 60 65 70 75
Days after pollination

FIG. 22

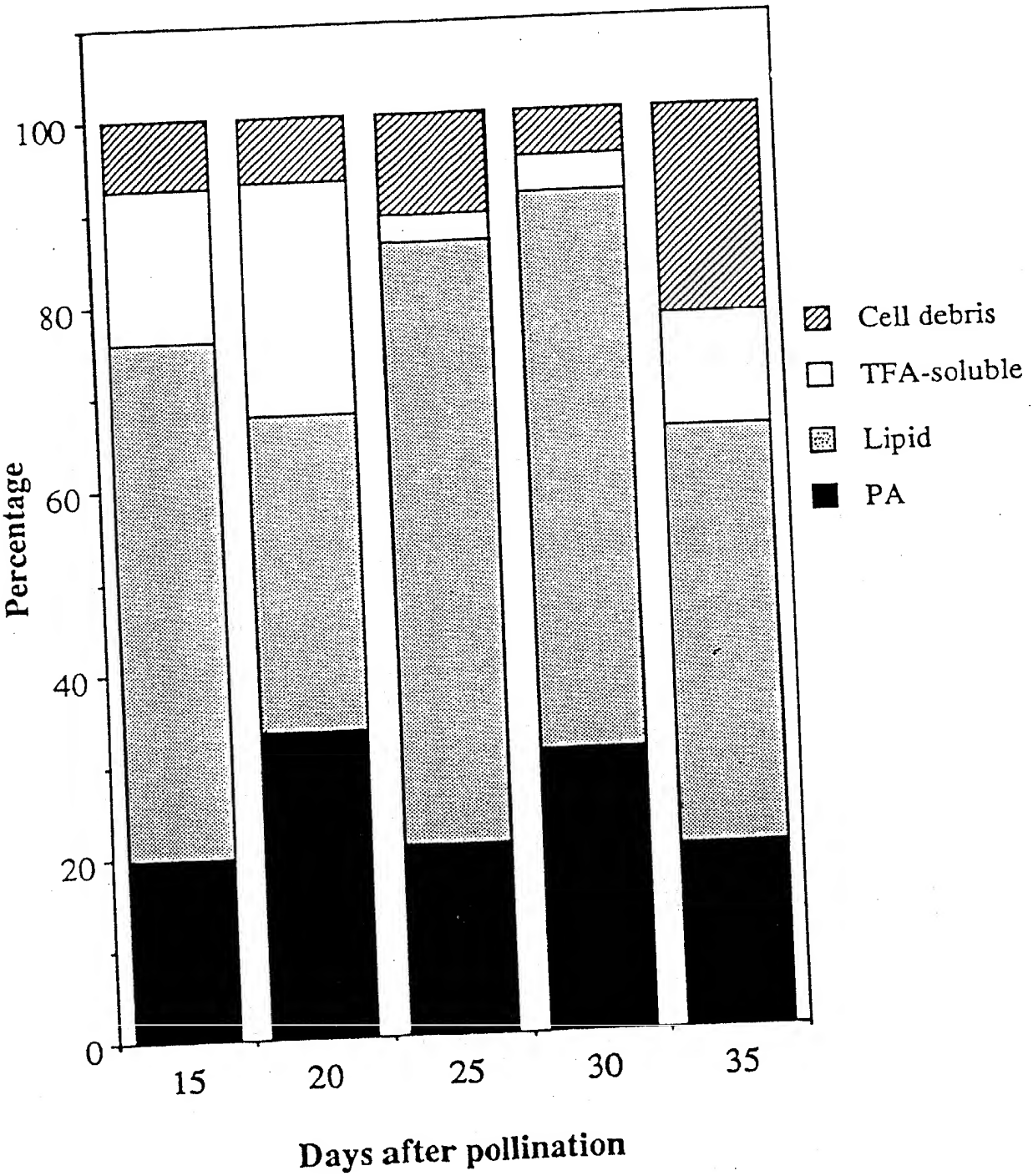

100
80
60
40
20
0
Percentage
15
20
25
30
35
Days after pollination
Cell debris
TFA-soluble
Lipid
PA

FIG. 23

```
cipimtla : AAAAAAAAAATTTTACTTCTCTGTTTTACCAAAAAGAGAAAAAAAAAATGACTACTTACAC
   imtsp : ..................................................----------

cipimtla : CAATGGCAACTACACACAACCAAAAACCCTAGACAAAGATGAACAATTAGCTGGTTTGGC
   imtsp : ------------------------------------------------------------

cipimtla : AGTGACATTAGCAAATGCAGCTGCTTTTCCAATGATCCTGAAATCAGCCTTTGAGCTAAA
   imtsp : ------------------------------------------------------------

cipimtla : AATCCTTGACATATTCTCAAAAGCAGGGGAAGGCGTGTTTGTATCGACTTCTGAGATCGC
   imtsp : ------------------------------------------------------------

cipimtla : TAGCCAAATCGGGGCAAAGAACCCTAATGCCCCGGTGTTGTTGGACCGGATGCTCCGGCT
   imtsp : ------------------------------------------------------------

cipimtla : CCTGGCTAGCCACTCTGTGTTAACATGCAAGCTCCAAAAGGGTGAGGGTGGTTCTCAAAG
   imtsp : ------------------------------------------------------------

cipimtla : GGTGTATGGTCCAGCTCCCCTTTGCAACTATCTTGCTAGTAATGATGGTCAAGGCTCTCT
   imtsp : ------------------------------------------------------------

cipimtla : TGGCCCTTTGCTTGTTTTGCATCATGACAAGGTCATGATGGAGAGTTGGTTTCACTTGAA
   imtsp : ------------------------------------------------------------

cipimtla : TGATTACATACTAGAAGGAGGTGTTCCATTCAAGCGCGCTCATGGGATGATCCAATTCGA
   imtsp : ------------------------------------------------------------

cipimtla : CTACACTGGGACTGATGAAAGGTTCAATCATGTGTTCAACCAAGGGATGGCACACCACAC
   imtsp : ------------------------------------------------------------

cipimtla : TATCCTGGTCATGAAGAAGCTCCTTGACAACTACAATGGGTTTAATGATGTCAAGGTCCT
   imtsp : ------------------------------------------------------------

cipimtla : AGTTGATGTGGGTGGTAACATTGGTGTCAATGTGAGCATGATCGTCGCTAAGCATACTCA
   imtsp : ------------------------------------------------------------

cipimtla : CATTAAGGGCATCAACTATGACTTGCCTCATGTCATTGCTGATGCTCCTTCTTACCCCGG
   imtsp : ------------------------------------------------------------

cipimtla : TGTGGAGCATGTTGGTGGTAACATGTTTGAGAGCATACCACAAGCAGATGCCATTTTCAT
   imtsp : ------------------------------------------------------------

cipimtla : GAAGTGGGTGTTGCATGATTGGAGCGACGAGCATTGCGTGAAGATACTCAACAAGTGCTA
   imtsp : ------------------------------------------------------------

cipimtla : TGAGAGCCTGGCAAAGGGAGGGAAGATCATCCTTGTGGAATCGCTTATACCAGTAATCCC
   imtsp : ------------------------------------------------------------

cipimtla : AGAAGACAACCTCGAATCACACATGGTGTTTAGCCTTGATTGCCACACTTTGGTGCACAA
   imtsp : ------------------------------------------------------------
```

FIG. 24

```
cipimt1a : CCAAGGTGGAAAAGAGAGATCAAAGGAGGATTTTGAAGCCTTAGCTTCCAAGACTGGCTT
   imtsp : ------------------------------------------------------------

cipimt1a : CTCTACAGTTGATGTCATTTGCTGTGCCTATGACACTTGGGTCATGGAGCTCTACAAGAA
   imtsp : ------------------------------------------------------------

cipimt1a : GTGATTCAAGCTCTAAATGCTGTGTTGTTGTCATTGTTGCTAGCCCAAGTAGCTAGCTAG
   imtsp : ------------------------------------------------------------

cipimt1a : CTGGTTAAAATTTCTCCTACCTAGCATTTGTTTTATGGCTAAGTTGAGGAGATTCATGTA
   imtsp : ------------------------------------------------------------

cipimt1a : TTGTAAATGTTGTGTTTGGGTTTGGGTTTGTATTTGTATTTGTGTTTTGTTGTTGTGTCT
   imtsp : ------------------------------------------------------------

cipimt1a : TTGTAGCTAAGTTGATATCCTGCTCATCTAGGCTGGCTGCATTTTTTTTGTGGCTGCCTG
   imtsp : ------------------------------------------------------------

cipimt1a : ACAATGTAGCATTTGTGGTTTTCTTTCAATAAAGCATCTA.TTGTACCTCTGTTATCAGT
   imtsp : -G----------------------------------------t-------------------

cipimt1a : GTATGATTTGCCTTTATTTTTAATAACTTAATTTTTTTTTTCTTGTTTATATCCA
   imtsp : -----------------.....................................
```

FIG. 24
(continued)

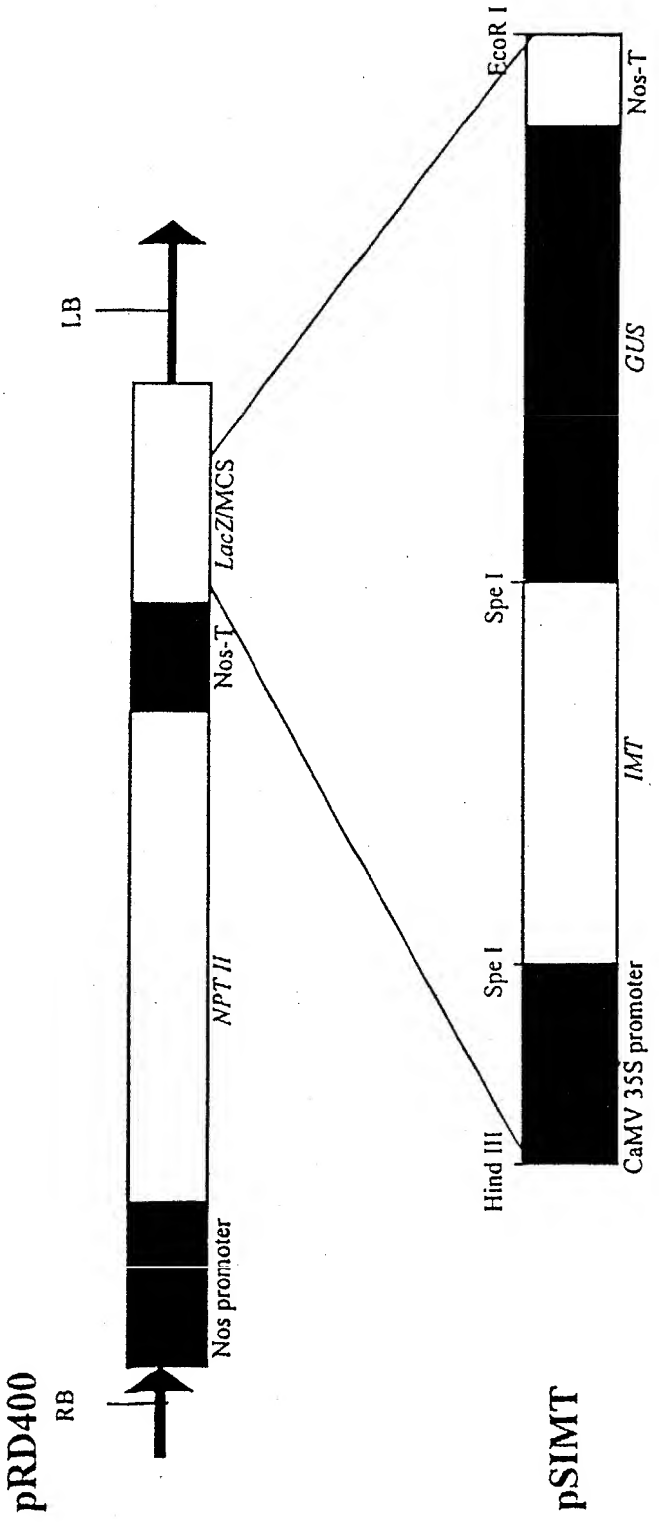

pRD400
RB
Nos promoter
NPT II
Nos-T
LacZ/MCS
LB
pSIMT
Hind III
Spe I
CaMV 35S promoter
IMT
Spe I
GUS
Nos-T
EcoR I

FIG. 25

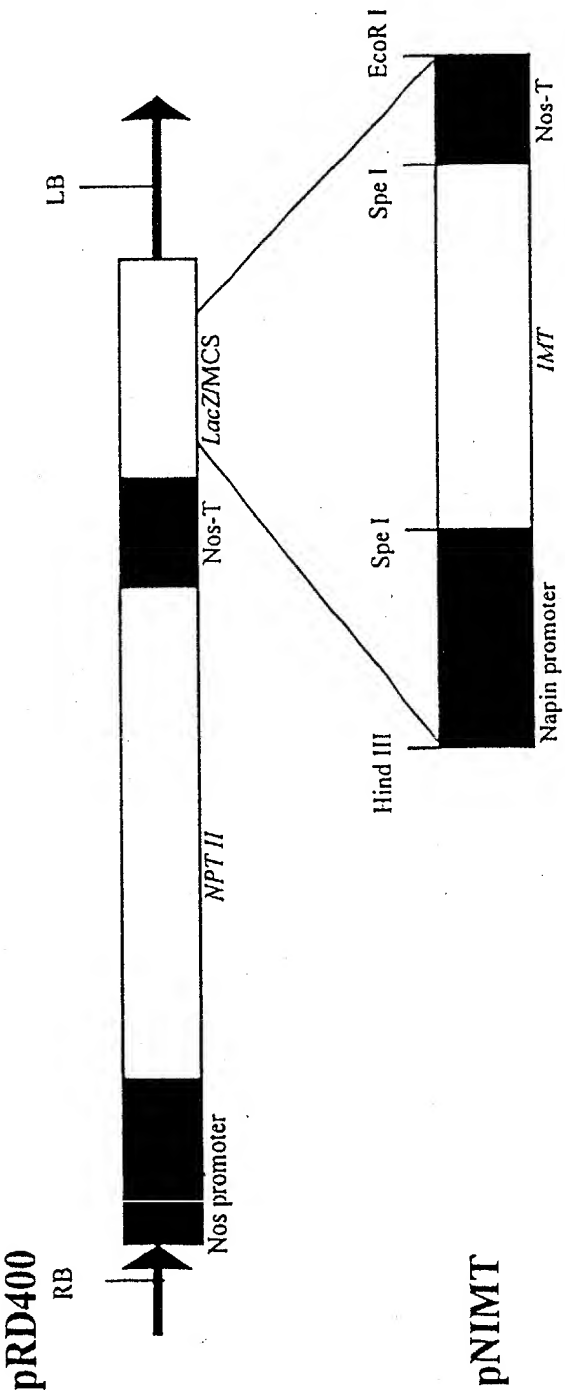
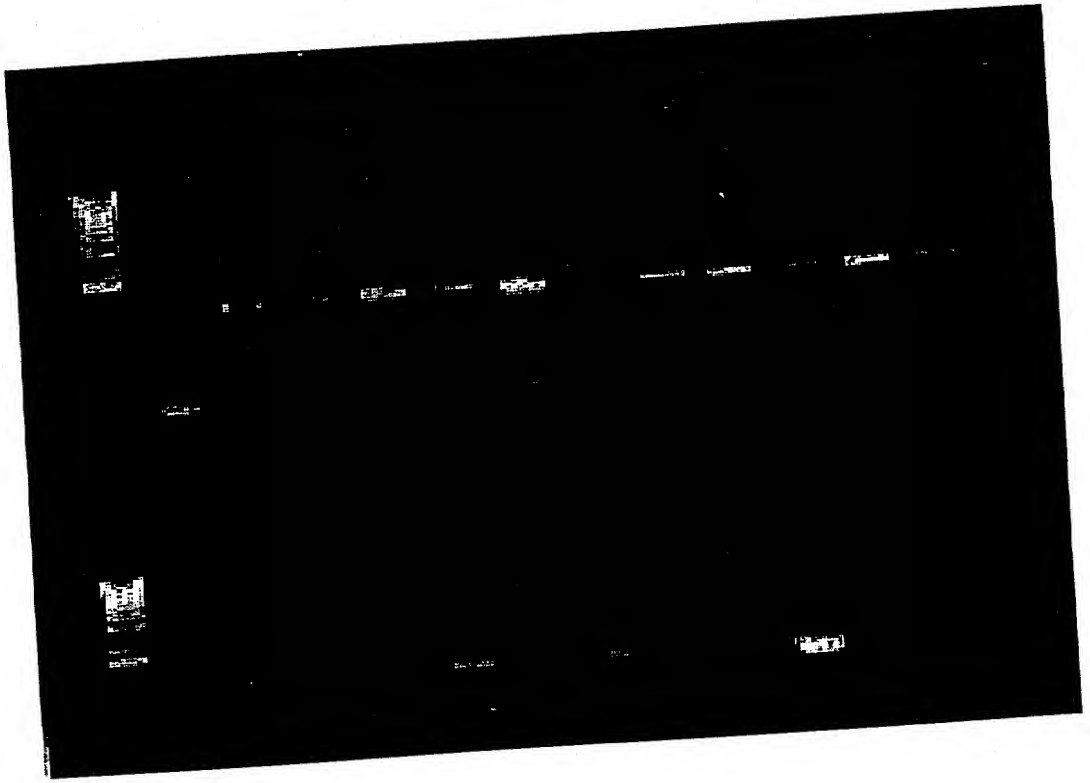

pRD400
RB
Nos promoter
NPT II
Nos-T
LacZ/MCS
LB
pNIMT
Hind III
Napin promoter
Spe I
IMT
Spe I
Nos-T
EcoR I

FIG. 26

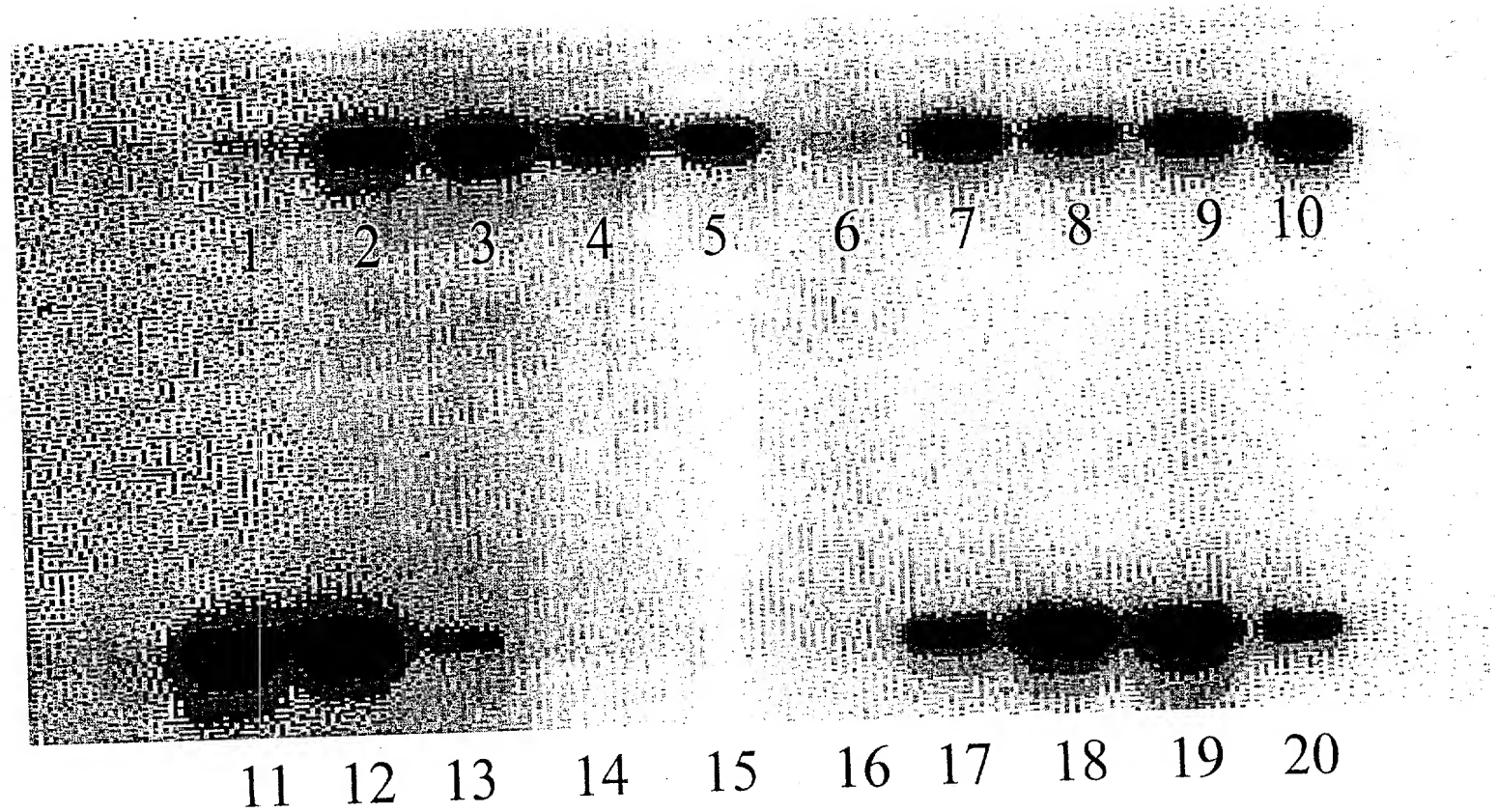
PCR analysis of transgenic plants containing the *IMT* gene



M=molecular marker ;1= control plant ;2-22=transgenic plants

Figure 27

Northern blot analysis of 35S-IMT transgenic plants



1-20=individual transgenic plants

FIG. 28

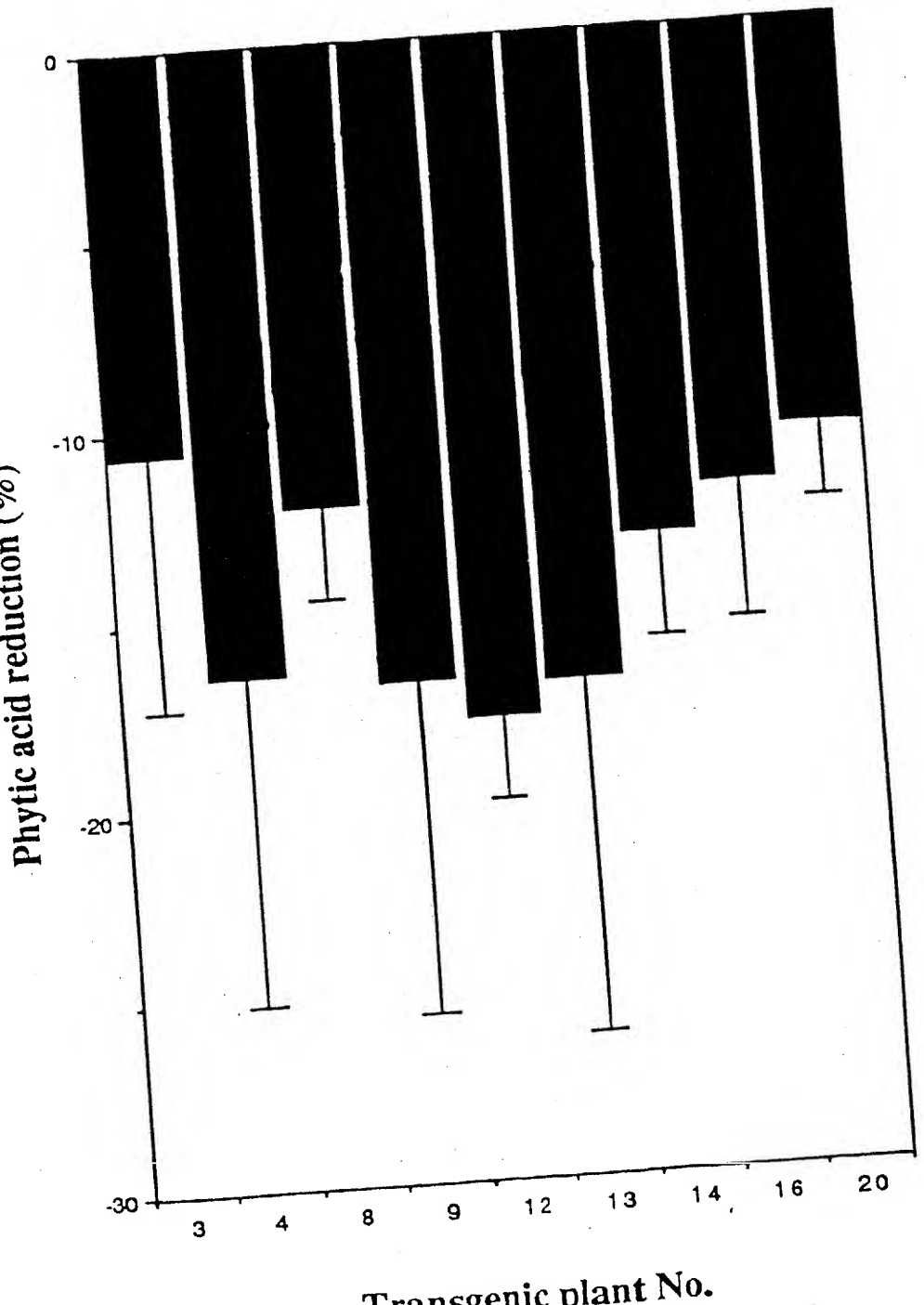

0
-10
-20
-30
Phytic acid reduction (%)
3
4
8
9
12
13
14
16
20
Transgenic plant No.

FIG. 29

Reduction of Phytic Acid in transgenic Plants

Percent Phytic Acid reduction in F1, F2 & F3 seeds from plants containing pSIMT

Transgenic Plant Number	Copy number of inserted gene	Percent Phytic Acid Reduction in F1 seeds	Percent Phytic Acid Reduction in F2 seeds	Percent Phytic Acid Reduction in F3 seeds
3	4	-10.5	-26	-36
6	3	-3.7	-24	-32
11	1	-8.6	-30	-34
17	3	-5.7	-29	-25

(a negative percent (-) means a reduction in phytic acid relative to non-transformed plants.)

FIG. 30

Reduction of Phytic Acid in transgenic Plants

Percent Phytic Acid reduction in F1 & F2 seeds from plants containing pNIMT

Transgenic Plant Number	Copy number of inserted gene	Percent Phytic Acid Reduction in F1 seeds	Percent Phytic Acid Reduction in F2 seeds
5	1	+24.31	-37
7	n.d.	-6.99	-44.81
12	2	-1.2	-39
15	1	-5.36	-24
17	2	-1.5	-38
19	3	-7.38	-43
21	1	-7.78	-37
N	1	+17.76	-31.54

(a negative percent (-) means a reduction in phytic acid relative to non-transformed plants.)

FIG. 31